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Chapter 2

***Chlamydia trachomatis*: identification of susceptibility markers for ocular and sexually transmitted infection by immunogenetics**

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Abstract

The aim of this review is to present a concise overview of all data available on the immunogenetics of *Chlamydia trachomatis* infections, both sexually transmitted urogenital and ocular infections. Currently, candidate gene approaches are used to identify genes related to the susceptibility to and severity of *C. trachomatis* infections. The main focus in the review will be on data obtained by the study of human cohorts

Introduction

Chlamydia trachomatis infection is the leading cause of blindness (trachoma) and the most prevalent sexually transmitted disease, strongly associated with pelvic inflammatory disease, ectopic pregnancy and tubal infertility. The prevalence of infection is increasing worldwide, with almost 100 million new infections each year (Starnbach & Roan, 2008). Some striking differences between individuals are observed in the clinical course of infection with *C. trachomatis*. In the case of sexually transmitted infection with *C. trachomatis* the following differences are observed.

Transmission vs. no transmission

Not all partners of a *C. trachomatis* positive index patient are themselves *C. trachomatis* positive. Transmission of the infection from the index patient to the partner is observed in between 45% and 75%, with lower rates of transmission from asymptomatic individuals (screening population) compared with those attending an STD clinic for symptoms.^{12,31,32}

Symptomatic vs. asymptomatic course of infection

The registered infections are mainly symptomatic, with people consulting a physician due to clinical symptoms and complaints. However, it is known that *C. trachomatis* can also run an asymptomatic course of infection in 80% of women and 50% of men.^{29,35}

Persistence vs. clearance of infection

In some people the infection clears spontaneously, whereas in others there is persistent infection for years. Some of the treated infections seem to reappear despite cotreatment of the partners.^{9,10,16,17,34}

Development of late complications (such as tubal infertility) vs. no development of late complications

Chlamydia trachomatis infection can ascend to the upper genital tract, resulting in pelvic inflammatory disease, ectopic pregnancy and tubal infertility. Uncontrolled immune reactions in the tubae are believed to contribute to the disease pathogenesis. Repeated infections are associated with the development of these late complications. However, only some women develop secondary complications after infection.^{10,17,34}

Ocular *C. trachomatis* infection causes inflammatory changes in the conjunctiva, and repeated infections sometimes lead to fibrosis and scarring of the subtarsal conjunctiva. This may cause the upper eyelid margin to turn inwards, causing the lashes to rub against the eyeball (trichiasis), which damages the cornea and leads ultimately to blindness. However, a subgroup of individuals develop more severe and persistent clinical disease in response to infection and are more likely to develop conjunctival scarring and trichiasis in later life. The reasons for this heterogeneity in susceptibility to *Chlamydial* infection and disease progression, following a rather uniform bacterial exposure, remain incompletely understood. In general these differences in the clinical course of infection can be explained by the interaction between the host (host factors) and the pathogen (virulence factors). This interaction is influenced by environmental factors such as coinfections. Although some studies have shown relationships between *C. trachomatis* serovars¹⁴⁻¹⁶ and the clinical course of infection¹⁶ and differences in infection variables between serovars have been described¹³, at present no clear single bacterial virulence factor has been identified that is related to the aforementioned differences in the clinical course of infection.

If the cellular immune response to *C. trachomatis* is subject to genetic influences, then the degree and mechanisms of such genetic control may have important implications for understanding the immunopathogenesis of *C. trachomatis* infection, therapeutic strategies and vaccine development, all of which are necessary to effectively treat and prevent *C. trachomatis* infection.

Chlamydia twin studies

It is clear that there are major interindividual differences in the susceptibility to and severity of infectious diseases. The best known example is malaria, which is caused by *Plasmodium* spp. People who are heterozygous for haemoglobin S (HbS) are protected against infection with *Plasmodium falciparum*, whereas those homozygous mutant for HbS have sickle cell anaemia.

Twin studies have advanced the efforts to identify susceptibility genes to infectious diseases. Comparison of concordance rates in monozygotic and dizygotic twins provides an estimate of the size of the genetic component of susceptibility, and for many infectious diseases this is substantial.

Recently, Bailey *et al.* (2009) published the most relevant study in the field of *Chlamydia* Immunogenetics, which was presented at the Ninth International Symposium on Human *Chlamydia* Infections in Napa, CA, in 1998. They estimated the relative contribution of host genetics to the total variation in lymphoproliferative responses to *C. trachomatis* antigen by analysing these responses in 64 Gambian pairs of twins from trachoma-endemic areas. Proliferative responses to serovar A EB antigens were estimated in monozygotic and dizygotic twin pairs. They found a stronger correlation and lower within-pair variability in

these responses in monozygotic compared with dizygotic twin pairs. The heritability estimate was 0.39, suggesting that host genetic factors contributed almost 40% of the variation.

Candidate gene approaches: single nucleotide polymorphisms (SNPs)

Candidate gene analyses are conceptually the simplest approach to a complex disease trait like infectious diseases. The selection of genes can be based on mRNA expression studies, protein profiling, animal studies including knockout models, and data obtained in similar infections (in the case of *C. trachomatis*, for instance, tuberculosis). In addition, often a logical selection of potential candidate genes is made on the basis of biological knowledge of the infection. For instance, *C. trachomatis* has lipopolysaccharide in its membrane, and the Toll-like receptor 4 (TLR4) is a lipopolysaccharide-sensing receptor on the outside of antigen-presenting cells and on epithelial cells, making this a potentially relevant candidate gene. As *C. trachomatis* is also present inside cells, selection of intracellular receptors involved in the recognition of molecular patterns present in *C. trachomatis* makes sense: for instance, TLR9, which recognizes CpG island in bacteria. Once genes have been selected, SNPs have to be identified, making use of published studies of those genes in other (infectious) diseases and using online databases, including dbSNP, the SNPper site and HapMap. Preferentially, functional SNPs have to be selected: SNPs that have a proven effect on the transcription and/or translation, resulting in higher or lower expressions of mRNAs and protein. The most widely used analysis is whether the frequency of a specific genetic variant is significantly different between diseased individuals and healthy controls (susceptibility analyses). An example is comparing *C. trachomatis* DNA positive individuals with *C. trachomatis*-negative individuals, correcting for potential confounding factors. Another possibility is to compare *C. trachomatis* positive patients with a different course of infection (severity analysis), for example comparing *C. trachomatis* positive women who develop tubal pathology with those who do not, or patients with an ocular *C. trachomatis* infection who develop conjunctival scarring and trichiasis in later life, with those who do not. Statistical analyses are often simple, making use of χ^2 testing or similar statistical approaches. The most important variables to generate reliable data in these kinds of candidate gene approaches are:

- (1) clear ethnic background definition of the population studied, as the incidence of SNP differs between different ethnic populations: for instance, the TLR4+896 A > G SNP occurs in 9% of Caucasians, whereas it is nonexistent in people from the orient;
- (2) clinical definition of disease: how is *C. trachomatis* positivity defined, and how are tubal pathology and ocular severity defined? Major differences in *C. trachomatis* diagnostics are present, as is the case for tubal pathology definition.

Data obtained by candidate gene approaches for *C. trachomatis*

Pathogen recognition receptors (PRRs)

PRRs are the first line of defence against invading pathogens. These receptors are an integral part of the innate immune system and alterations of their function or expression may affect the immune response.

Several members of the TLR family, CD14, NOD2, CCR5, and MBL, have been studied in relation to *C. trachomatis* pathogenesis (see Fig. 1 and Tables 1 and 2). TLR4, TLR9, CD14, and NOD2 were not associated with *Chlamydia* infection or with tubal pathology in single gene analyses; however, women carrying two or more mutations in these genes were at increased risk of developing tubal pathology following *Chlamydia* infection. *Chlamydial* lipopolysaccharide is a relatively weak TLR4 stimulus; we have shown, however, that with other TLR SNPs it modifies the risk of developing tubal pathology. This can partly be due to the fact that *Chlamydial* heat-shock protein 60 (HSP60) can also respond to TLR4 and human HSP60, potentially resulting in autoimmune-based tubal pathology, a mechanism described frequently in the literature. This process of TLR stimulation by nonpathogen-derived patterns is called sterile inflammation.

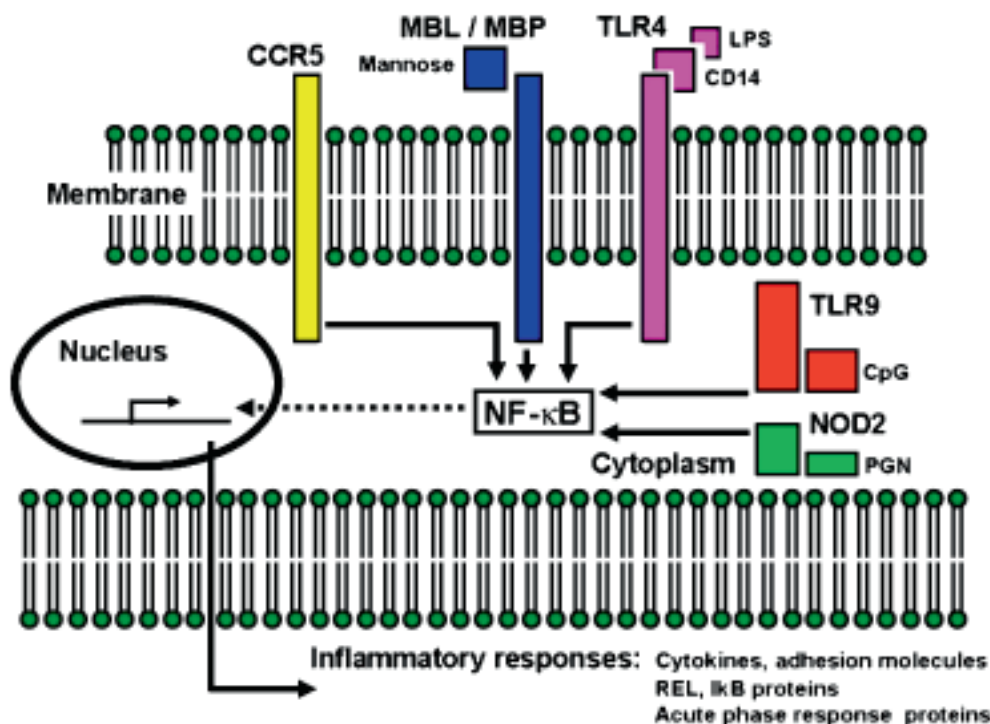


Figure 1. Overview of pathogen recognition receptors associated with *Chlamydia trachomatis* pathogenesis.

Both CCR5 and MBL were associated with late complications of *Chlamydia* infections (Table 2). The two most interesting findings were the MBL mutant allele in tubal pathology ($P < 0.001$)³⁰ and the role of CCR5 in tubal pathology, which was also underlined by corresponding KO murine studies.²

Cytokines

Cytokines are involved in a wide range of biological processes (Table 1) and have an important immunoregulatory function. Changes in expression or functionality of these cytokines may result in a dysregulated immune response.

The SNPs studies in interleukin-1B (IL-1B) and its receptor antagonist tumour necrosis factor- α (TNF- α), transforming growth factor β , interferon- γ (IFN- γ) and IL-6 are not associated with tubal infertility. In addition, no associations were observed between IL-2, IL-4, IL-4R, IL-6 and IL-12B and *Chlamydia* infections (Table 3). IL-10 was associated with tubal pathology, but only when in combination with specific HLA-DQB alleles.

Different SNPs in the TNF- α , IL-10, IFN- γ and IL-4 genes have been studied in relation to ocular *Chlamydia* infections. Several SNPs are associated with either scarring trachoma or trachiasis; however, some results, especially in the IL-10 haplotypes, seem contradictory (Table 3) in part because different IL-10 SNPs and haplotypes were studied in different ethnic populations.

IL-4 SNPs were not found to be associated with either urogenital or ocular *Chlamydia* infections, indicating that this gene may not be involved in *Chlamydia* pathogenesis.

IFN- γ was found to be associated with ocular infection but not with urogenital infections, indicating site-specific differences in the immune response to *Chlamydia*.

In summary, IL-10 SNPs and haplotypes have been associated with tubal infertility ($P = 0.005$;¹¹) scarring trachoma and trachiasis, for example in scarring trachoma ($P = 0.009$;¹⁹) (Table 3).

Table 1: Genes used in immunogenetic studies of *Chlamydia* infections, their biological functions and location in the genome.

Gene	Biological effect	Chromosome
<i>Pathogen recognition receptors</i>		
TLR4	TLR4, in complex with CD14, has been implicated in signal transduction events induced by lipopolysaccharide (LPS) found in most gram-negative bacteria. Mutations in this gene have been associated with differences in LPS responsiveness.	9q32-q33
TLR9	TLR9 mediates cellular response to unmethylated CpG dinucleotides in bacterial DNA to mount an innate immune response. It is localized and acts in an intracellular compartment. CpG DNA induces a strong T-helper-1-like inflammatory response.	3p21.3
CD14	CD14 acts as a co-receptor for TLR4 and TLR2, and confers responsiveness to LPS, a component of the cell wall of most Gram-negative bacteria. CD14 forms a complex with LPS and the LPS-binding protein (LBP). Combined with TLR4 this complex induces NF- κ B associated immune responses including the release of a broad spectrum of cytokines that include tumour necrosis factor alpha (TNF- α), IL-1, IL-6, and IL-8 to initiate immune response	5q31.1
NOD2	NOD2 is a member of the Nod1/Apaf-1 family and encodes a protein with two caspase recruitment (CARD) domains and six leucine-rich repeats (LRRs). The protein is primarily expressed in the peripheral blood leukocytes. It plays a role in the immune response to intracellular bacterial lipopolysaccharides (LPS) by recognizing the muramyl dipeptide (MDP) derived from them and activating the NF κ B protein. Mutations in this gene have been associated with Crohn disease and Blau syndrome.	16q12
CCR5	Potential role for the chemokine receptor in granulocyte lineage proliferation and differentiation. Chemokine receptor CCR5, a principal HIV-1 coreceptor, is posttranslationally modified by O-linked glycosylation and by sulfation of its N-terminal tyrosines. Sulfated tyrosines contributed to the binding of CCR5 to MIP-1-alpha, MIP-1-beta, and HIV-1 gp120/CD4 complexes, and to the ability of HIV-1 to enter cells expressing CCR5 and CD4. Mycobacterial heat-shock protein 70 (HSP70), in addition to enhancing antigen delivery to human dendritic cells, signals through the CCR5 chemokine receptor, promoting dendritic cell aggregation, immune synapse formation between dendritic cells and T cells, and the generation of effector immune responses.	3p21
MBL / MBP	This gene encodes the soluble mannose-binding lectin or mannose-binding protein found in serum. The protein encoded belongs to the collectin family and is an important element in the innate immune system. The protein recognizes mannose and N-acetylglucosamine on many microorganisms, and is capable of activating the classical complement pathway. Deficiencies of this gene have been associated with susceptibility to autoimmune and infectious diseases.	10q11.2-q21

Gene	Biological effect	Chromosome
<i>Cytokines</i>		
IL1B	IL1 is involved in a wide variety of physiological processes, including the regulation of inflammatory, metabolic, haematopoietic and immunological mechanisms. It is produced by macrophages, neutrophils and endothelial cells. IL1B initiates the expression of several genes coding for lymphokines. It induces natural killer cells and activates T and B cells	2q14
IL1RN	IL1RN specifically inhibits IL1 bioactivity on T cells and endothelial cells in vitro and is a potent inhibitor of IL1-induced corticosterone production in vivo. Interleukin-1 receptor antagonist levels are elevated in the blood of patients with a variety of infectious, immune, and traumatic conditions. IL1RN is expressed in the human beta cell and provides localized protection against leptin and glucose-induced islet IL1-beta.	2q14.2
IL2	Interleukin-2 (IL2) is a powerfully immunoregulatory lymphokine that is produced by lectin or antigen-activated T cells. It is produced not only by mature T lymphocytes on stimulation but also constitutively by certain T-cell lymphoma cell lines. It augments natural killer cell activity. It functions as growth factor for both B and T lymphocytes	4q26-q27
IL4	Interleukin-4 (IL-4) is a pleiotropic Th2-derived immune cytokine, which is predominantly produced by activated T lymphocytes, mast cells, and basophils. IL-4 has been shown to have various activities in many different cell types, such as T cells, B cells, monocytes, endothelial cells, and fibroblasts.	5q31.1
IL4R	IL4 is a cytokine produced by T cells that plays a major role in immunoglobulin E (IgE) production, and regulates proliferation and differentiation of a variety of cells. It modulates the activity of these cells following binding to its cell surface receptor, IL4R.	16p12.1-p11.2
IL6	IL6 is an immunoregulatory cytokine that activates a cell surface signaling assembly composed of IL6, IL6RA (IL6R), and the shared signaling receptor gp130 (IL6ST)	7p21
IL10	IL10 is an anti-inflammatory cytokine. It arrests and reverses the (chronic) inflammatory response.	1q31-q32
IL12B	IL12 is a proinflammatory cytokine. It has a broad range of biological functions, which include sustaining long term protection against intracellular pathogens.	5q31.1-q33.1
TNF α	TNF α is involved in the regulation of a wide spectrum of biological processes including cell proliferation, differentiation, apoptosis, lipid metabolism, and coagulation. This cytokine has been implicated in a variety of diseases, including autoimmune diseases, insulin resistance, and cancer. Knockout studies in mice also suggested the neuroprotective function of this cytokine.	6p21.3
LTA	Lymphotoxin alpha, a member of the tumor necrosis factor family, is a cytokine produced by lymphocytes. LTA is highly inducible, secreted, and exists as homotrimeric molecule. LTA forms heterotrimers with lymphotoxin-beta which anchors lymphotoxin-alpha to the cell surface. LTA mediates a large variety of inflammatory, immunostimulatory, and antiviral responses. LTA is also involved in the formation of secondary lymphoid organs during development and plays a role in apoptosis.	6p21.3

Gene	Biological effect	Chromosome
IFN γ	IFN- γ is secreted by Th1 cells, Tc cells, dendritic cells and NK cells. IFN- γ has antiviral, immunoregulatory, and anti-tumour properties. It increases antigen presentation of macrophages. IFN- γ activates and increases lysosome activity in macrophages and suppresses Th2 cell activity. It causes normal cells to express class II MHC molecules, and promotes adhesion and binding required for leukocyte migration. IFN- γ promotes NK cell activity.	12q14
TGF β	TGF-beta converts naive T cells into regulatory T cells that prevent autoimmunity. However, in the presence of IL6, TGF-beta also promotes the differentiation of naive T lymphocytes into proinflammatory IL17 cytokine-producing T helper-17 (Th17) cells, which promote autoimmunity and inflammation. vitamin A metabolite retinoic acid as a key regulator of TGF-beta-dependent immune responses, capable of inhibiting the IL6-driven induction of proinflammatory Th17 cells and promoting antiinflammatory regulatory T cell (Treg) differentiation.	19q13.1
<i>HLA</i>		
HLA-A / -B / -C / -DQA / -DQB / -DR	HLA / MHC genes are by far the most polymorphic of the human genome. The HLA proteins present antigens generated from proteins and present them to T cells. This presentation restricts the range of cellular and antibody responses to antigens.	6p21.3
<i>Other</i>		
MMP9	Involved in degradation of extracellular matrix molecules. MMP9 release might induce stem cell mobilization by cleaving matrix molecules to which stem cells are attached. MMP9 expression is related to aggressive tumour behaviour by induction/promotion of angiogenesis.	20q11.2-q13.1
I κ B-a	I κ B-a (NFKBIA) inactivates NF κ B by trapping in the cytoplasm, thus inhibiting proinflammatory signals.	14q13
I κ BL	This gene encodes a divergent member of the I-kappa-B family of proteins. Its function has not been determined. The gene lies within the major histocompatibility complex (MHC) class I region on chromosome 6.	6p21.3

Human leukocyte antigen (HLA)

The HLA system is a very versatile system able to recognize a variety of pathogens. Various HLA alleles have been linked to (infectious) disease pathogenesis. It is therefore not surprising that the scientific literature describes associations between HLA alleles and *Chlamydia* pathogenesis (see Table 4). Several HLA alleles have been associated with increased risk for urogenital *Chlamydia* infections and its late complications.

Similarly, associations have been found between HLA alleles and ocular *Chlamydia* infections. The strongest association was found by Conway *et al.* (1996) with HLA subtyping for allele A 6802, which was more frequent in cases of *C. trachomatis* infections as compared with controls ($P = 0.009$). Besides the HLA associations for urogenital tract and ocular *C. trachomatis* infections, HLA association has also been described for *C. trachomatis*-based reactive arthritis (ReA). The mechanisms that lead to the development of ReA are complex and basically involve an interaction between an arthritogenic agent and a predisposed host. The involvement of *C. trachomatis* in HLA-B27-associated ReA is well described.⁵ In addition, recently a *Chlamydia* positive Japanese man with Reiter's syndrome, negative for HLA-B27 or any other HLA-B27 cross-reactive major histocompatibility complex class I antigens, was positive for HLA-B51. It was therefore suggested that the combination of *Chlamydia* infection and HLA-B51 might play a role in the pathogenesis of Reiter's syndrome.²⁸

These results indicate that the HLA system has a profound impact on *Chlamydia* pathogenesis, which is not limited to specific ethnic populations.

Other approaches

Matrix metalloproteinases are involved in the turnover of the extracellular matrix, and through that process have been associated with disease processes. It has been shown that a specific SNP and a haplotype of MMP9 decrease the risk of trachomatous scarring and trachiasis with P-values of up to 0.006 (see Table 4).

Table 2: Immunogenetic association studies on *Chlamydia* infections focussed on pathogen recognition receptors

Gene	Polymorphism	Cohort	n	Ethnicity	Genotype freq. (%)	Results	Author
<i>Pathogen recognition receptors</i>							
<i>TLR4</i>	+896A>G (Asp299Gly)	Tubal Infertility	35	Dutch Caucasian	AA: 85.7 AG: 14.3 GG: 0.0	N.S.	Morré, 2003
<i>TLR4</i>	+896A>G (Asp299Gly)	Tubal Pathology	227	Dutch Caucasian	AA: 88.0 *G: 12.0	N.S., although increasing risk for Tubal Pathology was observed in trend analyses. Carriage of two or more SNPs in TLR9, TLR4, CD14, and CARD15/NOD2 increased the risk of developing Tubal Pathology following <i>Chlamydia</i> infection (N.S.)	Den Hartog, 2006
<i>TLR9</i>	-1237 T>C	Tubal Pathology	227	Dutch Caucasian	TT: 68.0 *C: 32.0	idem	Den Hartog, 2006
<i>TLR9</i>	+2848 G >A	Tubal Pathology	227	Dutch Caucasian	GG: 20.0 *A: 80.0	idem	Den Hartog, 2006
<i>CD14</i>	-260 C>T	<i>Chlamydia</i> infection / Tubal pathology	576 / 253	Dutch Caucasian	CC: 28.1 / 27.7 CT: 50.7 / 49.0 TT: 21.2 / 23.3	N.S.	Ouburg, 2005
<i>CD14</i>	-260 C>T	Tubal Pathology	227	Dutch Caucasian	CC: 26.0 *T: 74.0	N.S., although increasing risk for Tubal Pathology was observed in trend analyses. Carriage of two or more SNPs in TLR9, TLR4, CD14, and CARD15/NOD2 increased the risk of developing Tubal Pathology following <i>Chlamydia</i> infection (N.S.)	Den Hartog, 2006

Gene	Polymorphism	Cohort	n	Ethnicity	Genotype freq. (%)	Results	Author
<i>CARD15</i> / <i>NOD2</i>	SNP13 (Leu1007FsInsC)	Tubal Pathology	227	Dutch Caucasian	WT/WT: 93.0 *InsC: 7.0	idem	Den Hartog, 2006
<i>CCR5</i>	$\Delta 32$	Subfertility / Tubal Pathology	256	Dutch Caucasian	WT/WT: 80.0 WT/ $\Delta 32$: 19.5 $\Delta 32/\Delta 32$: 0.5	Decreased carriage of the <i>CCR5</i> deletion in women with tubal pathology and a positive <i>Chlamydia</i> serology, suggesting a protective effect of the deletion against <i>Chlamydia</i> induced tubal pathology.	Barr, 2005
<i>MBL</i>	Codon 54 (A>B)	Tubal Pathology	107	Hungarian Caucasian	AA: 54.6 AB: 31.9 BB: 13.5	Carriage of the mutant allele was significantly associated with tubal occlusions (P: <0.001; OR: 4.6; 95%CI: 2.3-8.9). Women with positive <i>Chlamydia</i> serology and tubal occlusions had the highest rates of B allele carriage (P: 0.001; OR: 3.9; 95%CI: 1.9-8.2). Allele B carriage was more frequent in <i>Chlamydia</i> serology negative women with blocked fallopian tubes compared to those with patent tubes (P: 0.01; OR: 3.5; 95%CI: 1.3-9.0)	Sziller, 2007
<i>MBP</i>	Codon 57 (Gly/ Glu)	Scarring trachoma	179	Gambian	Gly/Gly: 54.2 Gly/Glu: 39.7 Glu/Glu: 6.1	N.S.	Mozzato-Chamay, 2000

Abbreviations: CT: *Chlamydia trachomatis*; OR: Odds ratio; CI: Confidence interval; N.S.: Not significant; M.I.F.: Micro ImmunoFluorescence ; N/A: Not available; RO: Relative Odds; WT: Wildtype; MT: Mutant

Table 3: Immunogenetic association studies on *Chlamydia* infections focussed on cytokines

Gene	Polymorphism	Cohort	N	Ethnicity	Genotype freq. (%)	Results	Author
<i>Cytokines</i>							
<i>IL-1B</i>	-511 C>T	Tubal Factor Subfertility	40	Dutch Caucasian	CC: 40.0 CT: 52.5 TT: 7.5	N.S.	Murillo, 2003
<i>IL-1B</i>	+3954 C>T	Tubal Factor Subfertility	40	Dutch Caucasian	CC: 62.5 CT: 30.0 TT: 7.5	N.S.	Murillo, 2003
<i>IL-1RN</i>	86bp VNTR	Tubal Factor Subfertility	40	Dutch Caucasian	x.x: 60.0 x.2: 32.5 2.2: 7.5	N.S.	Murillo, 2003
<i>IL-2</i>	-330 T>G, 160 G>T (Haplotypes: G-G, T-G, T-T)	<i>Chlamydia</i> infection (Reach study)	485	North American (71% African American)	N/A	N.S.	Wang, 2005
<i>IL-4</i>	-590 T>C	Scarring trachoma	238	Gambian	TT: 50.0 TC: 38.7 CC: 11.3	N.S.	Mozzato-Chamay, 2000
<i>IL-4</i>	-1098 T>G, -590 C>T, -33 C>T (Haplotypes: T-T-T, T-G-C, T-C-C, G-C-C)	<i>Chlamydia</i> infection (Reach study)	485	North American (71% African American)	N/A	N.S.	Wang, 2005
<i>IL-4R</i>	1902 A>G	<i>Chlamydia</i> infection (Reach study)	485	North American (71% African American)	N/A	N.S.	Wang, 2005
<i>IL-6</i>	-174 G>C	Tubal Infertility	70 (35 MIF+ / 35 MIF-)	Kenyan	GG: 94.0 / 94.0 GC: 3.0 / 6.0 CC: 0.0 / 0.0	N.S.	Cohen 2003
<i>IL-6</i>	-174 G>C 565 G>A	<i>Chlamydia</i> infection (Reach study)	485	North American (71% African American)	N/A	N.S.	Wang, 2005
<i>IL-10</i>	-3575 T>A	Scarring trachoma / Trachiasis	651	Gambian	TT: 63.0 TA: 32.0 AA: 5.0	Associated with trachomatous scarring (P0.001; OR: 1.4; 95%CI: 1.1–1.7)	Natividad, 2005

Gene	Polymorphism	Cohort	N	Ethnicity	Genotype freq. (%)	Results	Author
<i>IL-10</i>	-1082 A>G	Tubal Factor Infertility	52	Finnish	AA: 22.0 AG: 41.0 GG: 37.0	N.S., however combined carriage with DQA1*0102 or DQB1*0602 with IL1-1082AA more frequent in cases than controls (P:0.005)	Kinnunen 2002
<i>IL-10</i>	-1082 A>G	Scarring trachoma	238	Gambian	AA: 44.1 AG: 42.4 GG: 13.5	G allele more frequent in Case than in controls in a ethnic subgroup (Mandinkas) (P:0.009; OR: 5.1; 95%CI: 1.2–24.2)	Mozzato-Chamay, 2000
<i>IL-10</i>	-1082 A>G	Scarring trachoma / Trachiasis	651	Gambian	AA: 46.0 AG: 41.0 GG: 0.13	G allele associated with scarring trachoma in the Mandinka ethnic group (P: 0.038, OR: 1.6; 95%CI: 1.1–2.4).	Natividad, 2005
<i>IL-10</i>	-819 C>T	Scarring trachoma	238	Gambian	CC: 29.8 CT: 46.2 TT: 24.0	N.S.	Mozzato-Chamay, 2000
<i>IL-10</i>	-592 A>C	Scarring trachoma	238	Gambian	AA: 24.0 AC: 47.9 CC: 28.1	N.S.	Mozzato-Chamay, 2000
<i>IL-10</i>	-592 A>C	Scarring trachoma / Trachiasis	651	Gambian	AA: 30.0 AC: 46.0 CC: 24.0	N.S.	Natividad, 2005
<i>IL-10</i>	+5009 A>G	Scarring trachoma / Trachiasis	651	Gambian	AA: 40.0 AG: 46.0 GG: 14.0	Associated with trachomatous scarring (P: 0.04; OR: 1.2; 95%CI: 1.0–1.5)	Natividad, 2005

Gene	Polymorphism	Cohort	N	Ethnicity	Genotype freq. (%)	Results	Author
<i>IL-10</i>	RS3024496 (3'UTR)	Scarring trachoma / Trachiasis	651	Gambian	WT/WT: 39.6 WT/MT: 45.0 MT/MT: 13.7	Longterm complications of trachomatous scarring and the severe phenotype of trachiasis increased with the number of mutant alleles (Ptrend: <0.001; ORtrend: 1.5; 95%CI: 1.3 -1.7; and Ptrend: <0.001; ORtrend: 1.7; 95%CI: 1.3-2.2)	Natividad, 2008
<i>IL-10</i>	-3575 T>A, -2763 C>A (Haplotypes: T-C, T-A, A-C, A-A)	<i>Chlamydia</i> infection (Reach study)	485	North American (71% African American)	N/A	N.S.	Wang, 2005
<i>IL-10</i>	-1082 A>G / -819 C>T / -592 A>C haplotypes	Tubal Infertility	70 (35 MIF+ / 35 MIF-)	Kenyan	GCC/GCC: 9.0 / 9.0 GCC/ACC: 20.0 / 6.0 GCC/ATA: 31.0 / 42.0 ACC/ACC: 9.0 / 0.0 ACC/ATA: 14.0 / 28.0 ATA/ATA: 14.0 / 14.0	N.S.	Cohen 2003
<i>IL-10</i>	-1082 A>G / -819 C>T / -592 A>C haplotypes	<i>Chlamydia</i> infection (Reach study)	485	North American (71% African American)	N/A	GCC haplotype negatively associated with recurrent <i>Chlamydia</i> infection (P: 0.04; RO: 0.6; 95%CI: 0.4-1.0)	Wang, 2005

Gene	Polymorphism	Cohort	N	Ethnicity	Genotype freq. (%)	Results	Author
<i>IL-10</i>	-3575 T>A / -1082 T>C / -592 G>T / +5009 A>G haplotypes	Scarring trachoma / Trachiasis	651	Gambian	TTTA: 48.1 TCGA: 7.3 TTGA: 7.8 ATTA: 0.1 ACGG: 19.2 TCGG: 6.7 ATGG: 2.3 TTGG: 8.5 TTTG: 0.0 ACGA: 0.1	ACGG and ATGG haplotypes associated with scarring trachoma (P: 0.045; OR: 1.3; 95%CI: 1.0–1.6; and P: 0.03; OR: 2.0; 95%CI: 1.1–3.7, respectively).. The TCGA haplotype was associated with protection against scarring trachoma (P: 0.048; OR: 0.7; 95%CI: 0.6–1.0).	Natividad, 2005
<i>IL12B</i> (<i>p40</i>)	1188 A>C (3' UTR)	<i>Chlamydia</i> infection (Reach study)	485	North American (71% African American)	N/A	N.S.	Wang, 2005
<i>TNF-α</i>	-308 G>A	Scarring trachoma	153	Gambian	GG: 71.6 GA: 24.1 AA: 4.2	Increased carriage of the AA genotype in patients compared to controls (P: 0.03; OR: 3.4; 95%CI: 0.7–17.1). Increased number of -308 or -238 mutants in patients than controls (x2 for trend: 8.6; P: 0.003). TNFα-308*A significantly associated with HLA A28, B70, Cw2, DRB1*11, and DRB1*1303 alleles in study subjects (P<0.006)	Conway, 1997

Gene	Polymorphism	Cohort	N	Ethnicity	Genotype freq. (%)	Results	Author
<i>TNF-α</i>	-308 G>A	Tubal Infertility	70 (35 MIF+ / 35 MIF-)	Kenyan	GG 86.0 / 83.0 GA: 6.0 / 17.0 AA: 6.0 / 0.0	N.S.	Cohen 2003
<i>TNF-α</i>	-308 G>A	Scarring trachoma / Trachiasis	651	Gambian	GG: 60.0 GA: 36.0 AA: 0.04	TNFA-308*A associated with trachiasis (P: 0.016; OR: 1.5; 95%CI: 1.1- 2.2).	Natividad, 2007
<i>TNF-α</i>	-376 G>A	Scarring trachoma	238	Gambian	GG: 94.5 GA: 5.5 AA: 0.0	N.S	Mozzato- Chamay, 2000
<i>TNF-α</i>	-238 G>A	Scarring trachoma	153	Gambian	GG: 83.9 GA: 14.1 AA: 2.1	Increased number of -308 or -238 mutants in patients than controls (x2 for trend: 8.6; P: 0.003). TNFa-238*A significantly associated with HLA B53, Cw5, Cw6, and DRB1*09 alleles in study subjects (P<0.0004)	Conway, 1997
<i>TNF-α</i>	-238 G>A	Scarring trachoma / Trachiasis	651	Gambian	GG: 87.0 GA: 13.0 AA: 0.07	N.S.	Natividad, 2007
<i>TNF-α</i>	-308 G>A, -238 G>A (Haplotypes: G-G, A-G, G-A)	<i>Chlamydia</i> infection (Reach study)	485	North American (71% African American)	N/A	N.S.	Wang, 2005
<i>LTA</i>	+72 G>T	Scarring trachoma / Trachiasis	651	Gambian	GG: 45.0 GT: 40.0 TT: 15.0	N.S.	Natividad, 2007
<i>LTA</i>	+252 A>G	Scarring trachoma / Trachiasis	651	Gambian	AA: 31.0 AG: 46.0 GG: 23.0	LTA+252*G associated with trachiasis (Ptrend: 0.018; OR: 1.4; 95%CI: 1.1- 1.8).	Natividad, 2007

Gene	Polymorphism	Cohort	N	Ethnicity	Genotype freq. (%)	Results	Author
<i>IFNg</i>	+874 T>A	Tubal Infertility	70 (35 MIF+ / 35 MIF-)	Kenyan	TT: 0.0 / 8.0 TA: 31.0 / 36.0 AA: 69.0 / 56.0	N.S	Cohen 2003
<i>IFNg</i>	-1616 C>T	Scarring trachoma / Trachiasis	651	Gambian	CC: 26.0 CT: 47.0 TT: 27.0	N.S.	Natividad, 2005
<i>IFNg</i>	+2200 T>C	Scarring trachoma / Trachiasis	651	Gambian	TT: 88.0 TC: 11.0 CC: 1.0	N.S.	Natividad, 2005
<i>IFNg</i>	+3234 T>C	Scarring trachoma / Trachiasis	651	Gambian	TT: 54.0 TC: 37.0 CC: 10.0	Associated with trachomatous scarring (P: 0.04; OR: 1.2; 95%CI: 1.0–1.5)	Natividad, 2005
<i>IFNg</i>	+5612 C>T	Scarring trachoma / Trachiasis	651	Gambian	CC: 49.0 CT: 41.0 TT: 10.0	N.S.	Natividad, 2005
<i>IFNg</i>	Haplotypes -1616 / +2200 / +3234 / +5612	Scarring trachoma / Trachiasis	651	Gambian	CTTC: 33.7 / CCTC: 6.4 CTTT: 8.9 / TTCC: 28.3 TTCT: 0.2 / TTTC: 0.5 TTTT: 21.9	TTCC associated with scarring trachoma (P: 0.02; OR: 1.3; 95%CI: 1.0–1.6)	Natividad, 2005
<i>TGF-β1</i>	Codon 10 T>C Codon 25 G>C	Tubal Infertility	70 (35 MIF+ / 35 MIF-)	Kenyan	TT-GG: 32.0 / 35.0 TC-GG: 27.0 / 29.0 TC-GC: 6.0 / 9.0 CC-GG: 29.0 / 18.0 TT-GC: 0.0 / 0.0 CC-GC: 3.0 / 9.0 CC-CC: 0.0 / 0.0 TT-CC: 0.0 / 0.0 TC-CC: 0.0 / 0.0	N.S.	Cohen 2003

Abbreviations: CT: *Chlamydia trachomatis*; OR: Odds ratio; CI: Confidence interval; N.S.: Not significant; M.I.F.: Micro ImmunoFluorescence ; N/A: Not available ; RO: Relative Odds; WT: Wildtype; MT: Mutant

Table 4: Immunogenetic association studies on *Chlamydia* infections focussed on HLA and other proteins

Gene	Polymorphism	Cohort	N	Ethnicity	Genotype freq. (%)	Results	Author
<i>HLA</i>							
<i>HLA</i>	DQA DQB	Tubal Factor Infertility & Tubal Ligation	47 & 46 (resp.)	Nairobi		DQA*0101 and DQB*0501 positively associated with CT tubal infertility (OR 4.9; 95%CI 1.3-18.6, and OR 6.8; 95%CI 1.6-29.2, resp.). DQA*0102 negatively associated with CT tubal infertility (OR 0.2; 95%CI 0.005-0.6).	Cohen 2000
<i>HLA</i>	DQA1 DQB1	Tubal Factor Infertility	52	Finnish		DQB1*0602 more frequent in cases compared to controls (p:0.04) . Combined carriage with DQA1*0102 or DQB1*0602 with IL1-1082AA more frequent in cases than controls (P:0.005)	Kinnunen 2002
<i>HLA</i>	DQA DQB DR	Tubal Infertility	70 (35 MIF+ / 35 MIF-)	Kenyan		HLA-DR1*1503 was more frequent in MIFwomen compared to MIF+ women (OR: 0.05; 95%CI: 0-0.7). DRB5*0101 was less common in MIF+ women than in MIFwomen (OR: 0.2; 95%CI: 0.02-1.0)	Cohen 2003
<i>HLA</i>	A B Cw DRB1 DQB1	Scarring trachoma	153	Gambian	A28: 25.8	The A28 allele was more frequent in cases than in controls (P: 0.046; OR: 1.9; 95%CI: 1.0-3.5). HLA subtyping found allele A*6802 more frequent in cases than controls (P: 0.009; OR: 3.1; 95%CI: 1.3-7.4).	Conway, 1996

Gene	Polymorphism	Cohort	N	Ethnicity	Genotype freq. (%)	Results	Author
<i>HLA</i>	A B C DRB1 DQB1	<i>Chlamydia</i> infection (Reach study)	485	North American (71% African American)		DRB1*03-DQB1*04 and DQB1*06 associated with recurrent <i>Chlamydia</i> infections (P < 0.01; RO > 2.0)	Wang, 2005
<i>HLA</i>	DQA DQB	PID (PEACH study) <i>Chlamydia</i> Cervicitis	92	American (2/3 'Black')	N/A/	Carriage of the DQA*0301 allele was more common among women with <i>Chlamydia</i> cervicitis (OR: 4.4 ; 95%CI: 1.6–12.0). Similar results were found for women carrying HLADQA*0501 (OR: 1.8; 95%CI: 0.7–4.9).	Ness, 2004
<i>Other</i>							
<i>MMP9</i>	rs2664538 A>G (Q279R)	Scarring trachoma / Trachiasis	651	Gambian	AA: 55.0 AG: 35.0 GG: 10.0	G allele associated with decreased risk for scarring trachoma and trachiasis (P: 0.012; OR: 0.7; 95%CI: 0.6-0.9; and P: 0.021; OR: 0.7; 95%CI: 0.5,-0.9). Heterozygotes (Q279R AG) were at lower risk of both TS and TT (P: 0.004; OR: 0.7; 95%CI: 0.5-0.8; and P: 0.006; OR: 0.6, 95%CI: 0.4-0.9, respectively).	Natividad, 2006
<i>MMP9</i>	rs2250889 C>G (R574P)	Scarring trachoma / Trachiasis	651	Gambian	CC: 73.0 CG: 25.0 GG: 2.0	N.S.	Natividad, 2006
<i>MMP9</i>	rs13969 A>C (G607G)	Scarring trachoma / Trachiasis	651	Gambian	AA: 35.0 AC: 46.0 CC: 20.0	N.S.	Natividad, 2006
<i>MMP9</i>	rs13925 G>A (V694)	Scarring trachoma / Trachiasis	651	Gambian	GG: 75.0 GA: 23.0 AA: 2.0	N.S.	Natividad, 2006

Gene	Polymorphism	Cohort	N	Ethnicity	Genotype freq. (%)	Results	Author
<i>MMP9</i>	Haplotype rs2664538 A>G / rs2250889 C>G / rs13969 A>C rs13925 G>A	Scarring trachoma / Trachiasis	651	Gambian	ACCG: 36.0 ACAG: 23.0 GCAA: 14.0 GCAG: 10.0 AGAG: 10.0 AGCG: 4.0 GCCG: 2.0	The risk of both TS and TT decreased with the number of copies of the haplotype GCAG (Ptrend: 0.07; OR: 0.8; 95%CI: 0.6-1.0; and Ptrend: 0.03; OR: 0.7; 95%CI: 0.5-1.0, for TS and TT respectively).	Natividad, 2006
<i>Ikb-a</i>	-881 A>G	Scarring trachoma	199	Gambian	AA: 94.5 AG: 5.0 GG: 0.5	The -881G/-826T haplotype was significantly decreased in cases compared to controls (P:0.046)	Mozzato-Chamay, 2001
<i>Ikb-a</i>	-826 C>T	Scarring trachoma	199	Gambian	CC: 94.5 CT: 5.0 TT: 0.5	idem	Mozzato-Chamay, 2001
<i>Ikb-a</i>	-297 C>T	Scarring trachoma	199	Gambian	CC: 98.0 CT: 2.0 TT: 0.0	N.S.	Mozzato-Chamay, 2001
<i>Ikb-a</i>	Haplotype -881/-826/-297	Scarring trachoma	199	Gambian	ACC: 95.0 GTC: 3.8 ACT: 0.5 GTT: 0.6	N.S.	Mozzato-Chamay, 2001
<i>IκBL</i>	-63 A>T	Scarring trachoma / Trachiasis	651	Gambian	AA: 30.0 AT: 47.0 TT: 22.0	IκBL -63*T associated with trachiasis (Ptrend: 0.004; OR: 1.5, 95%CI: 1.1-1.9).	Natividad, 2007

Gene	Polymorphism	Cohort	N	Ethnicity	Genotype freq. (%)	Results	Author
	Haplotype: IkBL-63 / LTA +77 / LTA +252 / TNF-308 / TNF-238	Scarring trachoma / Trachiasis	651	Gambian	ATAGG: 41.0 TGGGG: 22.0 TGGAG: 17.0 AGAGG: 11.0 AGAGA: 8.0 AGGGG: <1.0 AGGAG: <1.0 TGAGA: <1.0 TGAGG: <1.0 TTGGG: <1.0 TTAGG: <1.0	Two haplotypes (TGGGG and TGGAG) were independently associated with the risk for trachiasis (P: 0.005; OR: 1.6; 95%CI: 1.2-2.2; and P: 0.015; OR: 1.5; 95%CI: 1.1-2.2, respectively). The ATAGG haplotype was found to confer protection against trachiasis. Trend analyses showed that increasing number of the TGGGG haplotype increased the risk of trachiasis (Ptrend: 0.018; OR: 1.5; 95%CI: 1.1-2.0), while the ATAGG haplotype lowered trachiasis risk with increasing number of haplotypes (Ptrend: 0.012; OR: 0.75; 95%CI: 0.6-1.0)	Natividad, 2007

Abbreviations: CT: *Chlamydia trachomatis*; OR: Odds ratio; CI: Confidence interval; N.S.: Not significant; M.I.F.: Micro ImmunoFluorescence ; N/A: Not available ; RO: Relative Odds; WT: Wildtype; MT: Mutant

The IkBa and IkBL proteins are part of the inhibitory mechanism that reduces nuclear factor kB (NFkB) activation, thus limiting proinflammatory immune responses. IkBa SNPs reduce the risk of scarring trachoma, whereas IkBL SNPs confer a risk for trachiasis.

A haplotype spanning IkBL, lymphotoxin-a, and TNF-a confers both protection and risk for trachiasis, depending on the specific haplotype (Table 4).

In summary, current immunogenetic studies on *C. trachomatis* are slowly revealing in more detail that host genetic factors contribute almost 40% to the variation in responses to *C. trachomatis* between individuals. The clearest and most reproduced finding in ocular and sexually transmitted infection by *C. trachomatis* for susceptibility and severity factors is the role of HLA, IL-10 and traits of genetic variation in multiple genes including TLRs. Future studies will further pinpoint relevant genetic bio-markers. These studies are being substantiated by different types of data: (1) KO murine work (the relevance for TLR2, TLR4

and TLR9 has been presented at the Sixth Meeting of the European Society for *Chlamydia* Research) and forward genetics; (2) in vitro studies to assess the role of susceptibility genes in *C. trachomatis*–host interactions; (3) mRNA profiling in *C. trachomatis* and the human host to identify genes of interest; and (4) genomic-wide approaches in *C. trachomatis* and the human host to identify genes and regions of interest, relevant though costly approaches and the reason why candidate gene approaches are still very relevant.

Concluding remarks

There are several potential gains on a human health level to be achieved by immunogenetic studies of *C. trachomatis* infections: (1) further insight into the immunopathogenesis of *C. trachomatis* infections; (2) important implications for the understanding of *C. trachomatis*–host interactions; (3) identification of genetic markers of the susceptibility to and severity of *C. trachomatis* infections; (4) identification of these genetic markers can be used to develop diagnostic tools that can determine an individual's predisposition to infection and the risk to develop late complications; finally (5) these studies will allow the development of novel tools for the detection and treatment of, and vaccine development for, *C. trachomatis* infections.

Two major issues have to be addressed to maximize the output of the immunogenetic approaches for *C. trachomatis*: (1) the cohorts in which the current studies have been done are still (relatively) small and have to be extended both for ocular infections and sexually transmitted infections. One of the goals of the European Framework 6 funded EpiGenChlamydia Consortium (<http://www.EpiGenChlamydia.EU>) is to generate large cohorts in Europe and Africa. For this collection, biomedical ethical issues relating to the generation of multiethnic biobanks will need to be addressed properly. (2) Besides the candidate gene approaches, SNP chip approaches also have to be used to assess more genes and pathways, including those not addressed in the current candidate gene approaches. At the Sixth Meeting of the European Society of *Chlamydia* Research in Aarhus, Denmark, this July, three groups already showed preliminary work and the use of small dedicated SNP chips: the UK (London and Oxford) group of David Mabey, Robin Bailey and Dominic Kwiatkowski, the group of Deborah Dean (California) and our group (Amsterdam, the Netherlands).

These studies will provide new insights and new pathways to be studied, further advancing the exciting field of Immunogenetics of *C. trachomatis* infections.

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Statement

This is an invited MiniReview based on the Sixth Meeting of the European Society for *Chlamydia* Research.

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